A Study on Spontaneous Mutation to Bacteriophage Resistance in *Escherichia coli*, strain B

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Two generalized theories exist as explanations for the appearance of spontaneously arising mutant cells in a population of microorganisms. Such mutants could arise as a result of inexact gene replication during cell division or could be due to the mutagenic action of metabolic by-products which accumulate in the medium. No direct experimental approaches have been devised to test these two theories, although several indirect methods have been applied. The mutagenic metabolite theory can in part explain the results of experiments using the chemostat (6) in which cultures whose growth is regulated in a special steady-state, controlled-flow culture vessel mutate at nearly constant rates per unit time, whether the cells are multiplying rapidly or slowly. The duplication-mistake theory is substantiated partly by the work of Haas, *et al.* (4). They demonstrated that the incidence of spontaneously arising B/1 mutants of *Escherichia coli* was greater in chemically defined media than in more complex media. This assumes that more metabolic steps are necessary for gene synthesis in the simple substrates, and thus the chance for error in gene duplication becomes greater.

This latter work was based on the incidence of mutation rather than on mutation rate. Since this method of indicating mutagenic events does not have much meaning and since the various methods of calculating the rate of mutation (7) do not adequately circumvent selective factors, it was decided to repeat the work of Haas, *et al.*, by studying the growth curves of both the normal parent cells and the B/1 mutants to see if a clearer picture of the mutagenic events could be obtained.

Materials and Methods

The genetic marker used in these experiments was resistance of *Escherichia coli*, strain B, to the lytic action of bacteriophage T 1. One hundred ml. aliquots of media of complexity ranging from simple synthetic

to highly enriched were inoculated with from 10 to 3 x 10^8 cells per ml. Inocula of 10 to 100 cells per ml. (10^3 to 10^4 cells per aliquot) were usually free of B/1 mutants, or individuals resistant to phage T 1. The larger inocula were shown to contain an average of 7 B/1 cells.

Platings were made at hourly intervals until the stationary phase of growth was reached. The total viable count was determined by plating appropriate dilutions of the culture in nutrient agar and the number of B/1 mutants was determined by exposing samples of the culture to excess bacteriophage and plating using the agar layer technique (1).

Results and Discussion

By following the appearance and multiplication of the mutant cells together with the growth curve of the entire culture, it is possible to express results in terms of the mutation rate or incidence of mutation. The mutation rate can be calculated by any of the various formulae devised for this purpose which are not based on probability. The expression of the mutation rate is dependent upon the time of appearance of the mutant cells in the culture and upon the rate of multiplication of the mutant forms as well as the rate of formation of the mutants. The incidence of mutation is affected by the same factors if it is calculated during the logrithmic growth phase of the mutant population. However, if the calculation is made after logrithmic growth has ceased, the incidence becomes dependent upon the level of growth attained by the mutant population. Since none of these calculations adequately considers factors of selection due to population dynamics in the growing cultures, their actual meaning becomes obscure and does not express the probability of mutation per bacterium per physiological time unit or generation (2). Phenotypic lag (5) can also alter these calculations.

Some variation in the spontaneous mutation rate was observed in the various media used in these experiments. This variation was not uniformly correlated with the composition of the media, and it was found that small changes in the experimental procedure would change the mutation rate when using the same medium. Much of the data presented by Haas, *et al.* (4) can be explained as the result of suppression of the mutants by the metabolic activity of the parent culture. Grigg (3) has reported the suppression of mutants by large numbers of normal cells. The suppression found in these experiments was not due to total numbers of parent cells, but appeared to be due to their metabolic activity. Details on this suppression of mutant forms will be reported elsewhere.

Although the results of Haas, *et al.* were not verified, the theory of duplication error as a cause of spontaneous mutation cannot be discounted. It is possible that both postulated mechanisms are operative. Experimental verification of these theories cannot be satisfactory until factors of selection in a developing culture are better understood .

Summary

A study of the appearance of B/1 mutants of *Escherichia coli* in relation to the development of the parent culture revealed that there is no correlation between the spontaneous mutation rate of B/1 appearance and the complexity of the growth substrate. A suppression of the mutant cells by the metabolic activity of the parent culture was observed. This suppression can be misleading in the calculation and interpretation of mutation rates.

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